



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top; padding: 5px;"> <p>(21) International Application Number: PCT/EP00/00938</p> <p>(22) International Filing Date: 2 February 2000 (02.02.00)</p> <p>(30) Priority Data:</p> <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">60/118,295</td> <td style="width: 30%;">2 February 1999 (02.02.99)</td> <td style="width: 40%;">US</td> </tr> <tr> <td>60/118,235</td> <td>2 February 1999 (02.02.99)</td> <td>US</td> </tr> <tr> <td>60/118,282</td> <td>2 February 1999 (02.02.99)</td> <td>US</td> </tr> </table> <p>(71) Applicant (for all designated States except US): K.U. LEUVEN RESEARCH &amp; DEVELOPMENT [BE/BE]; Groot Begijnhof, Benedenstraat 59, B-3000 Leuven (BE).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): WAER, Mark, Jozef, Albert [BE/BE]; Waversebaan 263, B-3001 Heverlee (BE). HERDEWIJN, Piet, André, Maurits, Maria [BE/BE]; Olivierstraat 21, B-3111 Rotselaar/Wezemaal (BE). PFLEIDERER, Wolfgang, Eugen [DE/DE]; Lindauerstrasse 47, D-78464 Konstanz (DE).</p> <p>(74) Agent: PRINS, Hendrik, Willem; Arnold &amp; Siedsma, Sweelinckplein 1, NL-2517 GK The Hague (NL).</p> </td> <td style="width: 50%; vertical-align: top; padding: 5px;"> <p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b></p> <p><i>Without international search report and to be republished upon receipt of that report.</i></p> </td> </tr> </table>			<p>(21) International Application Number: PCT/EP00/00938</p> <p>(22) International Filing Date: 2 February 2000 (02.02.00)</p> <p>(30) Priority Data:</p> <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">60/118,295</td> <td style="width: 30%;">2 February 1999 (02.02.99)</td> <td style="width: 40%;">US</td> </tr> <tr> <td>60/118,235</td> <td>2 February 1999 (02.02.99)</td> <td>US</td> </tr> <tr> <td>60/118,282</td> <td>2 February 1999 (02.02.99)</td> <td>US</td> </tr> </table> <p>(71) Applicant (for all designated States except US): K.U. LEUVEN RESEARCH &amp; DEVELOPMENT [BE/BE]; Groot Begijnhof, Benedenstraat 59, B-3000 Leuven (BE).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): WAER, Mark, Jozef, Albert [BE/BE]; Waversebaan 263, B-3001 Heverlee (BE). HERDEWIJN, Piet, André, Maurits, Maria [BE/BE]; Olivierstraat 21, B-3111 Rotselaar/Wezemaal (BE). PFLEIDERER, Wolfgang, Eugen [DE/DE]; Lindauerstrasse 47, D-78464 Konstanz (DE).</p> <p>(74) Agent: PRINS, Hendrik, Willem; Arnold &amp; Siedsma, Sweelinckplein 1, NL-2517 GK The Hague (NL).</p>	60/118,295	2 February 1999 (02.02.99)	US	60/118,235	2 February 1999 (02.02.99)	US	60/118,282	2 February 1999 (02.02.99)	US	<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b></p> <p><i>Without international search report and to be republished upon receipt of that report.</i></p>
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<p>(54) Title: IMMUNOSUPPRESSIVE EFFECTS OF PTERIDINE DERIVATIVES</p> <div style="text-align: center; margin: 20px 0;"> <p style="margin-top: 10px;">(I)</p> </div> <p>(57) Abstract</p> <p>The invention relates to a pharmaceutical composition for the treatment of autoimmune disorders and/or for the treatment or prevention of transplant-rejections comprising a pteridine derivative of general formula (I) especially a lumazine optionally combined with a second active agent.</p>													

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## IMMUNOSUPPRESSIVE EFFECTS OF PTERIDINE DERIVATIVES

The invention relates to a pharmaceutical composition for the treatment of autoimmune disorders and/or the treatment or prevention of transplant-rejections comprising pteridine derivatives.

5           The invention further relates to combined pharmaceutical preparations comprising one or more pteridine derivatives and one or more known immunosuppressant, and to a group of novel pteridine derivatives as such.

10           Further the invention is also related to a method for the treatment of autoimmune disorders and/or of transplant-rejections.

          Several pteridine derivatives are known in nature and used in the preparation of medicines, for example as  
15   described in EP-A-108 890. Other medical uses of derivatives of pteridine are described in WO 95-31987 as NO-synthase inhibitors, for example for the treatment of diseases caused by a high nitrogen monoxide level. Further, WO-95-32203 describes also the use of  
20   tetrahydropteridine derivatives as NO-synthase inhibitors.

          Both above-mentioned WO publications disclose also the use of these specific pteridine derivatives in the treatment of pathologically low blood pressure, in  
25   particular septic shock and combined with cytokines in tumor therapy and in transplant-rejection diseases.

          Although some of these pteridine derivatives are claimed as potentially active for the treatment of transplant-rejection diseases, direct evidence for their  
30   effectiveness is lacking. Thus there still is a need for specific and highly active immunosuppressive compounds, in particular immunosuppressive compounds active in the cosignal pathway.

          A first object of the invention is to provide a  
35   pharmaceutical composition having high immunosuppressive activity. Another object of the invention is to provide a

combined immunosuppressive preparation which causes a superadditive effect, comprising a pteridine derivative of the invention and other known immunosuppressants.

Another further object of the invention is to  
5 provide immunosuppressive compounds, which are active in a minor dose, in order to decrease the considerable treatment costs.

Known immunosuppressive compounds are for example cyclosporine A, substituted xanthines, tacrolimus  
10 (FK 506), rapamycin (RPM), leflunomide, mofetil, adrenocortical steroids, cytotoxic drugs and antibody preparations.

The immunosuppressive effect of cyclosporine A (CyA) is already known since 1972. However, due to its  
15 nephrotoxicity and several other side effects CyA has not been able to establish itself as the optimal and final drug of choice.

Methylxanthines, for example pentoxifylline (PTX), are known having immunosuppressive effects in  
20 vitro.

Recently (Lin Y. et al, Transplantation 63 (1997) it has been found that the co-medication of an immunosuppressive compound such as cyclosporine A (CyA) or FK506 or RPM (rapamycin) with a methylxanthine  
25 derivative, in particular A802715 (7-propyl-1(5-hydroxy-5-methylhexyl)-3-methylxanthine) leads to a superadditive increase in the immunosuppressive action.

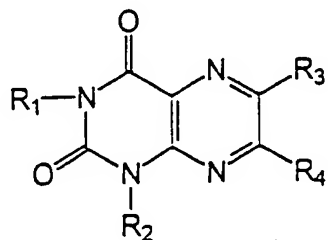
Likewise, other substituted, in particular substituted 8-phenylxanthines have been found to possess  
30 immunosuppressive effects in vitro (application EP 98.201323.7).

The present invention relates in particular to the application of a group pteridine derivatives and their pharmaceutical salts, possessing unexpectedly  
35 desirable pharmaceutical properties, i.e. are highly active immunosuppressive agents.

The invention demonstrates the

immunosuppressive effects of <sup>3</sup>pharmaceutical composition  
for the treatment of autoimmune disorders and/or for the  
treatment or prevention of transplant-rejections  
comprising a pteridine derivative of general formula:

5



10

wherein:

$R_1$  and  $R_2$  are independently hydrogen; aliphatic  
15 saturated or unsaturated; straight or branched carbon  
chain with 1 to 7 carbon atoms; substituted or  
unsubstituted aryl or alkylaryl substituents, whereby the  
carbon atoms may be oxidized represented by alcohol or  
carbonyl function or carboxylic acids and their esters;

20

$R_3$  and  $R_4$  are independently hydrogen, hydroxyl,  
halogen, alkyl, haloalkyl, alkoxy, wherein alkyl and the  
alkyl group may be branched or straight and contains one  
or four carbon atoms, formyl and derivatives such as  
25 hydroxylamino conjugates and acetals, cyano, carboxylic  
acids and carboxyl acid derivatives such as esters and  
amides, sulfhydryl, amino, alkylamino, cycloalkylamino,  
alkenylamino, alkynylamino, benzylamino,  
hydroxylalkylamino, morfolinoalkylamino, fenylhydrazino,  
30 morfoline, piperidine, mercaptobenzyl, mercaptoalkyl,  
cysteinyl ester, styryl, substituted or unsubstituted  
aromatic ring; aromatic or heterocyclic substituent  
substituted with an aliphatic spacer between the  
pteridine ring and the aromatic substituent of 1 to 4  
35 carbon atoms, whereby said spacer may contain an alcohol  
function, carbonyl function, halogen, ether, and may be  
saturated or unsaturated; branched or straight, saturated  
or unsaturated aliphatic chain of 1 to 7 carbon atoms

which may contain one or more functions chosen from the group comprising carbonyl, alcohol, ether, carboxyester, nitro, thioalkyl, halogen or a pharmaceutically acceptable salt thereof; and

5

X and Y are independently oxygen or sulfur or a pharmaceutical salt thereof, and a pharmaceutically acceptable carrier.

Preferred pteridine derivatives comprising  
10 compositions are given in claims 2-9. Particularly preferred are the compositions according to claim 10.

The invention further relates to a combined preparation having synergetic effects containing 1)  
15 cyclosporine A, substituted xanthines, tacrolimus (FK506), Rapamycin (RPM), Leflunomide, Mofetil, adrenocortical steroids, cytotoxic drugs and antibody compositions and 2) at least one pteridine derivative of formula (I) defined above, and optionally a  
20 pharmaceutical excipient, for simultaneous, separate or sequential use in (auto)immune disorders and/or in the treatment of transplant-rejections.

The invention further relates to a method for treating auto-immuno disorders or transplant-rejections in a subject by administering an effective amount of a  
25 pharmaceutical composition of claims 1-11, to the compounds as such as defined above, to the use of these compounds for the treatment of autoimmuno disorders and/or the treatment and/or prevention of transplant rejections, and to a method for selecting potent  
30 immunosuppressive agents based on the determination of the three parameters MLC, ACD<sub>1</sub> and ACD<sub>2</sub>.

Hereunder the effects of the pteridine derivatives on the lymphocyte activation are elucidated and are compared with standard reference compounds (see  
35 table I, compound 4, 6, 7, 11, 13, 19, 20, 21, 22, 25, 26, 28, 30, 34, 35).

Table I summarizes the tested compounds. These pteridine derivatives were obtained as follows:

#### 6-Bromomethyl-1,3-dimethylalumazine:

To a solution of 1,3,6-trimethylalumazine [1] (2.06 g, 0.01 moles) in glacial AcOH (60 ml) was added dropwise bromine (3.2 g, 0.02 moles) in AcOH (10 ml) and then  
5 heated under reflux for 1 hour. After cooling was evaporated, the residue dissolved in  $\text{CHCl}_3$  (100 ml), washed with  $\text{H}_2\text{O}$  (3 x 70 ml), the organic layer dried over  $\text{Na}_2\text{SO}_4$  and again evaporated. The residue was purified by silica gel column chromatography starting with  
10 toluene/EtOAc 9/1 to elute first 6-dibromomethyl-1,3-dimethylalumazine (1.49 g, 41%) and followed by toluene/EtOAc 4/1 to get 6-bromomethyl-1,3-dimethylalumazine. Yield: 1.2 g (42%). M.p. 228°C (decomp.). UV (MeOH): 244 (4.16); [264 (400)]; 337  
15 (3.86).

#### 7-Bromomethyl-1,3-dimethylalumazine:

Analogous to the preceding procedure with 1,3,7-trimethylalumazine [1] (2.06 g, 0.01 moles) and bromine  
20 (3.2 g, 0.02 moles) by heating for 2 hours. Isolation by silica gel column chromatography with toluene/EtOAc 9/1 to elute first 7-dibromomethyl-1,3-dimethylalumazine (2.07 g, 57%) and second 7-bromomethyl-1,3-dimethylalumazine. Yield: 0.97 g (34%). M.p. 165-166°C. UV (MeOH): 241  
25 (4.23); 338 (4.02).

#### 1,3-Dimethylalumazin-6-ylmethyl-triphenylphosphonium bromide:

To a suspension of 6-bromomethyl-1,3-dimethylalumazine  
30 (1.0 g, 3.5 mmol) in toluene (20 ml) triphenylphosphane (1.1 g, 4.2 mmol) was added and then heated at 80°C in an oilbath with stirring for 8 hours. After cooling the precipitate was collected, washed with EtOAc and dried at 100°C to give 1.8 g (94%) of a colorless powder of m.p.  
35 289°C. UV (MeOH): 204 (4.74); 227 (4.52); [243 (4.42)]; [262 (4.21)]; 338 (3.88).

1,3-Dimethyl-6-(E)-styryllumazine-7-ylmethyl-triphenylphosphonium  
bromide:

Analogous to the preceding procedure from 7-bromomethyl-  
1,3-dimethyl-6-(E)-styryllumazine and triphenylphosphane in toluene by  
5 heating under reflux for 1 day. Yield: 1.86 g (97%). M.p.  
261°C. UV (MeOH): 204 (4.76); [221 (4.54)]; 342 (4.09);  
414 (4.38).

General synthesis of 1,3-dimethyl-6-(E)-styryllumazines

10 1, 2, 3, 5:

To a solution of 1,3-dimethyl-6-(E)-styryllumazine-7-ylmethyl-  
triphenylphosphonium bromide and 1,3-dimethyl-6-(E)-styryllumazine-7-ylmethyl-  
triphenylphosphonium bromide (0.547 g, 1 mmole),  
respectively, in MeOH (5 ml) was added sodium methoxide  
15 (0.108 g, 2 mmoles) and stirred at room temperature for  
30 min. Then 1.5 mmoles of the aromatic or heteroaromatic  
aldehyde were added and stirring continued for 5 hours.  
The resulting precipitate was filtered off, washed with  
MeOH and purified by recrystallization from DMF/H<sub>2</sub>O to  
20 give a yellowish powder.

1,3-Dimethyl-6-(E)-styryllumazine (1):

According to the general procedure with benzaldehyde  
(0.16 g). Yield: 0.124 g (42%). M.p. 238°C. UV (MeOH):  
25 [220 (4.17)]; 308 (4.42); 372 (4.03).

1,3-Dimethyl-6-[(E)-2-(pyrid-3-yl)vinyl]lumazine (2):

According to the general procedure with pyridine-3-  
carboxaldehyde (0.162 g). Yield: 0.195 g (66%). M.p.  
30 210°C. UV (MeOH): [236 (3.92)]; 308 (4.29); 370 (3.97).

1,3-Dimethyl-6-[(E)-2-(pyrid-4-yl)vinyl]lumazine (3):

According to the general procedure with pyridine-4-  
carboxaldehyde (0.162 g). Yield: 0.156 g (53%). M.p.  
35 262°C. UV (MeOH): 202 (4.20); [238 (3.92)]; 307 (4.51);  
370 (4.20).

6-(1,2-Dibromo-2-phenylethyl)-1,3-dimethyl-6-(E)-styryllumazine (4):

To a solution of 1,3-dimethyl-6-(E)-styryllumazine (1) (0.735 g, 2.5 mmol) in  $\text{CHCl}_3$  (20 ml) was added bromine (0.8 g, 5 mmol) dissolved in  $\text{CHCl}_3$  (5 ml) and then the mixture stirred at room temperature for 4 hours. It was  
5 evaporated to dryness and the residue treated with MeOH to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield: 1.067 g (94%). M.p. 176°C. UV (MeOH): 245 (4.18); [260 (4.10)]; 341 (3.83).

10

1,3-Dimethyl-6-[(E)-4-(phenyl)butadienyl]lumazine (5): According to the general procedure with cinnamaldehyde (0.2 g). Yield: 0.138 g (43%). M.p. 252°C (decomp.). UV (MeOH): 228 (4.02); [244 (3.97)]; 330 (4.66); 389 (4.23).

15

6-[(E)-2-methoxycarbonylethenyl]lumazine:

To a suspension of methoxycarbonylmethyl-triphenylphosphonium bromide (0.415 g, 1 mmol) in dioxane (3 ml) was added DBU (0.23 g, 1.5 mmol) and  
20 stirred at room temperature for 30 min. Then 1,3-dimethyl-6-carboxaldehyde [1] (0.2 g, 0.91 mmol) was added and stirring continued for 5 hours. The precipitate was collected, washed with MeOH and dried to give a colorless crystal powder. Yield: 0.158 g (63%).  
25 M.p. 211-213°C (decomp.). UV (MeOH): 202 (4.46); [256 (4.14)]; 286 (4.21); 348 (4.08).

6-(1,2-Dibromo-2-(methoxycarbonyl)ethyl)-1,3-dimethyl-6-[(E)-2-methoxycarbonylethenyl]lumazine (6):

30 To a solution of 6-[(E)-2-methoxycarbonylethenyl]lumazine (0.7 g, 2.53 mmol) in  $\text{CHCl}_3$  (20 ml) was added bromine (0.64 g, 4 mmol) dissolved in  $\text{CHCl}_3$  (5 ml) and then the mixture stirred at room temperature for 6 hours. It was evaporated to dryness and the residue treated with MeOH  
35 to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield: 0.97 g (88%). M.p. 163°C. UV (MeOH): 247 (4.16); [260 (4.08)]; 339 (3.88).

6-(2-Bromo-2-methoxycarbonyl-ethenyl)-1,3-dimethylumazine (7):

To a solution of 6 (0.1 g, 0.23 mmoles) in dioxane (20 ml) was added DBU (70 MG, 0.43 g, 0.43 mmoles) and then stirred at room temperatur for 2 hours. It was diluted with ethyl acetate (100 ml), washed with H<sub>2</sub>O (3 x 50 ml), the organic layer separated, dried over Na<sub>2</sub>SO<sub>4</sub> and then evaporated. The residue was treated with MeOH, the solid collected and purified by recrystallization from DMF to give a yellowish powder. Yield: 0.055 g (68%). M.p. 204°C. UV (MeOH): [254 (4.08)]; 285 (4.25); 360 (4.01).

6-Chlorocarbonyl-1,3-dimethylumazine:

A suspension of 1,3-dimethylumazine-6-carboxylic acid [2] (3.0 g, 12.7 mmoles) in dry toluene (80 ml) was treated with freshly distilled thionyl chloride (50 ml) under reflux for 3 hours. It was evaporated to dryness, the residue treated with dry ether, the solid collected, washed with ether and dried in a vaccuum desiccator. Yield: 3.13 g (93%). M.p. 262-264°C. UV (dioxane): 256 (4.08); [280 (4.00)]; 333 (4.03).

6-[(2-Acetyl-2-ethoxycarbonyl)acetyl]-1,3-dimethylumazine (8):

A solution of ethyl acetoacetate ethoxy-magnesium salt [3] (0.8 g, 4 mmoles) in THF (8 ml) was added dropwise to a suspension of 6-chlorocarbonyl-1,3-dimethylumazine (0.51 g, 2 mmoles) in THF (10 ml) and then the mixture stirred at room temperature for 3 days. It was evaporated and the residue treated with cold 1 N HCl (20 ml, 0-5°C). The precipitate was collected, washed with H<sub>2</sub>O and dried in a vacuum desiccator. Purification was achieved by column chromatography (silica gel 3.5 x 12 cm) with CHCl<sub>3</sub>/MeOH 95/5 and the first main fraction collected. After evaporation was recrystallized from toluene (12 ml) to give colorless crystals. Yield: 0.247 g (36%). M.p. 153-156°C. UV (pH 2.0): 251 (4.09); 293 (4.10); 330 (4.11).

6-[2,2-(Diethoxycarbonyl)acetyl]-1,3-dimethylumazine  
(9):

To a solution of ethylmalonate ethoxy-magnesium salt [4]  
(0.685 g, 3 mmol) in THF (12 ml) was added 6-  
5 chlorocarbonyl-1,3-dimethylumazine (0.51 g, 2 mmol)  
and then the mixture stirred at room temperature for 20  
hours. It was evaporated, the residue treated with 1 N  
HCl (20 ml) and the resulting solid collected.  
Recrystallization from EtOH (40 ml) gave yellowish  
10 crystals. Yield: 0.585 g (78%). M.p. 124-126°C. UV (pH  
2.0): 253 (4.05); 291 (4.08); 332 (4.04).

6-(1-Methoxy-2-methoxycarbonyl)ethenyl)-1,3-  
dimethylumazine (10):

15 A suspension of 6 (0.2 g, 0.46 mmol) in dry MeOH (8 ml)  
was treated with a solution of sodium (0.046 g, 2 mmol)  
in MeOH (2 ml) at room temperature with stirring for 15  
min. Then  $\text{NH}_4\text{Cl}$  (0.1 g) and  $\text{H}_2\text{O}$  (10 ml) were added and the  
mixture extracted with  $\text{CHCl}_3$  (2 x 50 ml). The organic  
20 layer was dried over  $\text{Na}_2\text{SO}_4$ , evaporated and the residue  
crystallized from  $\text{CHCl}_3$ /n-hexane. Yield: 0.085 g (60%).  
M.p. 160°C. UV (MeOH): 204 (4.20); 245 (4.15); 288  
(4.23); 350 (3.99).

25 1,3-Dimethyl-6-[(2-nitro)ethenyl]umazine (11):

A solution of 12 (0.562 g, 2 mmol) in pyridine (10 ml)  
was cooled to 0°C and then acetic anhydride (4 ml)  
dropwise added. Cooling was removed and the mixture  
stirred at room temperature for 3 hours. The resulting  
30 precipitate was collected, washed with  $\text{H}_2\text{O}$  and dried in a  
vacuum desiccator to give a chromatographically pure  
product. Yield: 0.515 g (98%). Crystallization from  $\text{CHCl}_3$ .  
M.p. 232-234°C. UV (MeOH): [239 (3.63)]; 309 (3.87); 365  
(3.95).

35

6-[(1-Hydroxy-2-nitro)ethyl]-1,3-dimethylumazine (12):

To a solution of nitromethane (0.61 g, 10 mmol) and  
triethylamine (1.44 g, 10 mmol) in MeOH (20 ml) was

added 6-formyl-1,3-dimethylalumazine [1] (2.0 g, 9 mmol) and then the mixture stirred at room temperature for 5 hours. The precipitate was collected, washed with MeOH and ether and dried. Yield: 2.22 g (78%).

- 5 Recrystallization from  $\text{CHCl}_3$ . M.p. 166-167°C. UV (MeOH): 240 (4.38); 336 (3.98); [347 (3.89)].

- 6-[(1-Ethylthio-2-nitro)ethyl]-1,3-dimethylalumazine (13):  
To a suspension of compound 11 (0.263 g, 1 mmol) in MeOH  
10 (5 ml) and  $\text{H}_2\text{O}$  (5 ml) was added ethylmercaptane (0.093 g, 1.5 mmol) and DBU (0.2 g) and then the mixture stirred at room temperature for 1 hour. The precipitate was collected, washed and dried. Yield: 0.25 g (77%). M.p. 88°C. UV (MeOH): 203 (4.28); 240 (4.26); [262 (4.05)];  
15 341 (3.89).

6-Hydroxymethyl-1,3-dimethylalumazine (14) [1]:

- 1,3-Dimethyl-7-[(E)-2-(pyrid-2-yl)vinyl]alumazine (15):  
20 According to the general procedure with pyridine-2-carboxaldehyde (0.162 g). Yield: 0.233 g (79%). M.p. 282-283°C. UV (MeOH): 203 (4.14); 238 (4.23); 312 (3.95); 375 (4.36).

- 25 1,3-Dimethyl-7-[(E)-2-(pyrid-3-yl)vinyl]alumazine (16):  
According to the general procedure with pyridine-3-carboxaldehyde (0.162 g). Yield: 0.195 g (66%). M.p. 264-265°C. UV (MeOH): 208 (4.45); 234 (4.43); [274 (4.09)]; 307 (4.08); 375 (4.48).

- 30 1,3-Dimethyl-7-[(E)-2-(pyrid-4-yl)vinyl]alumazine (17):  
According to the general procedure with pyridine-4-carboxaldehyde (0.162 g). Yield: 0.215 g (73%). M.p. 307-310°C. UV (MeOH): 207 (4.12); 229 (4.01); 282 (3.79);  
35 [296 (3.76)]; 372 (4.00).

1,3-Dimethyl-7-[(E)-4-(phenyl)butadienyl]alumazine (18):

According to the general procedure with cinnamaldehyde (0.2 g). Yield: 0.195 g (61%). M.p. 277-287°C (decomp.). UV (MeOH): 239 (3.79); 299 (3.66); 402 (4.15).

5 7-[1,2-Dibromo-2-(methoxycarbonyl)ethyl]-1,3-dimethylalumazine (19):

To a suspension of 7-[(E)-2-methoxycarbonylethenyl]lumazine (1.79 g, 6.5 mmol) in CHCl<sub>3</sub> (70 ml) was added bromine (0.7 g, 14 mmol) dissolved in CHCl<sub>3</sub> (10 ml) and  
10 then the mixture stirred at room temperature for 3 hours. It was evaporated to dryness and the residue treated with MeOH to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield: 2.34 g (77%). Crystallization from  
15 EtOAc/n-hexane. M.p. 144-145°C. UV (MeOH): 240 (4.14); 343 (3.90).

7-[(E)-2-methoxycarbonylethenyl]lumazine (20):

To a suspension of methoxycarbonylmethyl-  
20 triphenylphosphonium bromide (0.415 g, 1 mmol) in dioxane (5 ml) was added DBU (0.23 g, 1.5 mmol) and stirred at room temperature for 30 min. Then 1,3-dimethylalumazine-7-carboxaldehyde [1] (0.2 g, 0.91 mmol) was added and stirring continued for 20 hours.  
25 The precipitate was collected, washed with MeOH and dried to give a colorless crystal powder. Recrystallization from DMF. Yield: 0.15 g (60%). M.p. 242-245°C (decomp.). UV (MeOH): 201 (4.21); 225 (4.29); 252 (4.20); 364 (4.11).

30

7-(1,2-Dibromo-2-phenylethyl)-1,3-dimethylalumazine (21):

To a solution of 1,3-dimethyl-7-(E)-styryllumazine (23) (0.735 g, 2.5 mmol) in CHCl<sub>3</sub> (20 ml) was added bromine (0.48 g, 3 mmol) dissolved in CHCl<sub>3</sub> (5 ml) and then the  
35 mixture stirred at room temperature for 3 hours. It was evaporated to dryness and the residue treated with MeOH to give a colorless precipitate. The solid was collected, washed with MeOH and dried in a vacuum desiccator. Yield:

1.08 g (95%). M.p. 187-188°C. UV (MeOH): 241 (4.25); 341 (4.06).

7-(1-Bromo-2-phenyl)ethenyl-1,3-dimethylalumazine (22):

5 To a suspension of 21 (0.2 g, 0.44 mmol) in dry MeOH (4 ml) was added a solution of sodium (0.05 g, 2.2 mmol) in MeOH (1 ml) and then the mixture stirred at room temperature for 3 hours. The precipitate was collected, washed with MeOH and dried in vacuum. Yield: 0.117 g  
10 (71%). Yellowish powder from DMF. M.p. 245-246°C. UV (MeOH): 243 (4.15); 372 (4.15).

7-Benzyl-1,3-dimethylalumazine:

A solution of 5,6-diamino-1,3-dimethyluracil  
15 monohydrochloride [5] (2.06 g, 0.01 mole) in H<sub>2</sub>O (50 ml) was treated with benzylglyoxal [6] (2.22 g, 0.015 moles) in EtOH (20 ml) and heated under reflux for 1 hour. It was diluted with H<sub>2</sub>O (50 ml) and then extracted with CHCl<sub>3</sub> (5 x 100 ml). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>,  
20 evaporated and the residue purified by silica gel column chromatography with toluene/EtOAc 10/1. The main fraction was collected, evaporated and crystallized from EtOH. Yield: 1.7 g (61%). M.p. 147-148°C. UV (MeOH): 238 (4.22); 332 (4.07).

25

1,3-Dimethyl-6-(E)-styryllumazine (23):

According to the general procedure with benzaldehyde (0.16 g). Yield: 0.223 g (76%). M.p. 259-260°C. UV (MeOH): 203 (4.17); 237 (4.11); 379 (4.29).

30

7-Benzoyl-1,3-dimethylalumazine (24):

A suspension of 7-benzyl-1,3-dimethylalumazine (0.56 g, 2 mmol) in H<sub>2</sub>O (30 ml) was treated with KMnO<sub>4</sub> (0.6 g) and heated under reflux for 30 min. After cooling was  
35 extracted with CHCl<sub>3</sub> (3 x 100 ml), the organic layer dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated to dryness. Crystallization from dioxane/H<sub>2</sub>O. Yield: 0.5 g (84%). M.p. 190-191°C. UV (MeOH): 233 (4.23); [255 (4.10)]; 347 (3.97).

7-Chloro-1,3-dimethylumazine (25) [7].

1,3-Dimethyl-7-mercaptopumazine (26) [8].

5 1,3-Dimethyl-6,7-diphenylumazine (27) [9].

1,3-Dimethyl-6-phenyl-7-mercaptopumazine (28):

A mixture of 7-hydroxy-1,3-dimethyl-6-phenylumazine [5] (2.84 g, 0.01 mole) and  $P_4S_{10}$  (3.3 g) was heated in  
10 pyridine (75 ml) under reflux for 1 hour. After cooling was diluted with  $H_2O$  (50 ml) and after standing for several hours the yellow precipitate (28-pyridinium salt, 3.3 g, 87%). The salt was dissolved in hot  $H_2O$  (100 ml) and acidified by HCl to pH 0. The resulting yellow  
15 crystals were collected, washed and dried in the oven. Yield: 2.22 g (74%). M.p. 145°C (decomp.). UV (MeOH): 203 (4.37); 227 (4.36); [283 (3.86)]; 370 (4.05).

7-Methoxy-1,3-dimethyl-6-phenylumazine (29):

20 A solution 7-hydroxy-1,3-dimethyl-6-phenylumazine [5] (1.42 g, 0.005 moles) in 0.5 N NaOH (20 ml) and MeOH (10 ml) was treated with dimethyl sulfate (1 ml) and stirred for 1 hour at room temperature. The resulting precipitate was collected, washed and dried in the oven. Yield: 1.26  
25 g (81%). M.p. 194°C. UV (MeOH): 205 (4.53); [240 (4.08)]; 281 (4.22); 343 (4.23).

7-Chloro-1,3-dimethyl-6-phenylumazine (30):

A mixture of 7-hydroxy-1,3-dimethyl-6-phenylumazine [5] (2.84 g, 0.01 mole) and  $NH_4Cl$  (1 g) was heated in  $POCl_3$ ,  
30 under reflux for 36 hours. It was evaporated to a syrup, ice was added and stirred with a glass rod till a precipitate was formed. The solid was collected, washed with  $H_2O$ , dried and then recrystallized from MeOH. Yield:  
35 2.36 g (78%). M.p. 180°C. UV (MeOH): 204 (4.47); 249 (4.23); 273 (4.24); 350 (4.05).

6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (31a):

A solution of 6-benzoyl-1,3-dimethylumazine (0.2 g, 0.68 mmol) in dry 1,2-dichloroethane (20 ml) was treated  
5 with  $\text{AlCl}_3$  (0.4 g, 3 mmol) and freshly distilled anisole (10 ml, 92 mmol) at room temperature and stirred for 24 hours. Then ice (50 g) was added, the aqueous phase extracted with  $\text{CHCl}_3$  (3 x 50 ml), the organic phase washed with 2%  $\text{NaHCO}_3$  solution (50 ml) and  $\text{H}_2\text{O}$  (50 ml), dried  
10 over  $\text{Na}_2\text{SO}_4$  and evaporated in high vacuum to remove excess of anisole. The residue was treated with toluene (50 ml) to obtain a yellow precipitate. Recrystallization from  $\text{EtOH}/\text{H}_2\text{O}$  1/1 gave yellow crystals. Yield: 0.176 g (65%). M.p. 240-244°C (decomp.). UV (MeOH): 254 (4.25); [270  
15 (4.21)]; 406 (4.08).

6-Benzoyl-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (31):

A suspension of 6-benzoyl-7,8-dihydro-1,3-dimethyl-7-(4-methoxyphenyl)lumazine (31a) (0.3 g, 0.74 mmol) in  
20 dioxane (40 ml) was treated at room temperature with 1%  $\text{KMnO}_4$  solution (10 ml) by dropwise addition with stirring. After 30 min the excess of  $\text{KMnO}_4$  was reduced by  $\text{NaHSO}_3$ , the  $\text{MnO}_2$  filtered off, washed with warm  $\text{EtOH}$  (3 x 20 ml) and then the united organic phases evaporated to dryness.  
25 The residue was purified by silica gel chromatography with  $\text{CHCl}_3/\text{MeOH}$  (25/1). The main fraction was collected, evaporated and the solid recrystallized from  $\text{EtOAc}$  with charcoal. Yield: 0.175 g (59%). M.p. 255-257°C. UV (MeOH): 253 (4.24); 367 (4.10).

30

6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-phenylumazine (32a):

Analogous to procedure 31a from 6-benzoyl-1,3-dimethylumazine (0.2 g, 0.68 mmol) and benzene (15  
35 ml). Yield: 0.21 g (83%). UV (MeOH): 254 (4.26); 407 (4.12).

6-Benzoyl-1,3-dimethyl-7-phenylumazine (32):

Analogous to procedure 31 from 6-benzoyl-7,8-dihydro-1,3-dimethyl-7-phenyllumazine (32a) (0.3 g, 0.78 mmol).  
Yield: 0.18 g (62%). M.p. 185-187°C. UV (MeOH): 252 (4.39); [290 (4.08)]; 349 (4.16).

5

7-Methoxy-1,3-dimethyl-6-styryllumazine (33):

To a suspension of compound 4 (0.2 g, 0.44 mmol) in dry MeOH (6 ml) was added DBU (0.2 ml, 1.34 mmol) and then stirred at room temperature for 2 hours. The precipitate  
10 was filtered off, washed with MeOH and dried in a vacuum desiccator. Yield: 0.134 g (94%). Crystallization from DMF. M.p. 271-272°C. UV (MeOH): [232 (4.11)]; 306 (4.36); 375 (4.38).

15 1-Methyl-6,7-diphenyllumazine (34) [10].

7-Hydroxy-3-methyl-6-phenyllumazine (35) [5].

7-Hydroxy-1,6-diphenyllumazine (36).

20 To a suspension of 6-diamino-5-nitroso-1-phenyluracil [11] (2.32 g, 0.01 mol) in H<sub>2</sub>O (50 ml) and EtOH (20 ml) was reduced catalytically with PtO<sub>2</sub>/H<sub>2</sub> in a shaking apparatus till about 450 ml of hydrogen was consumed. The mixture was heated, the catalyst filtered off and the  
25 filtrate treated with ethyl phenylglyoxylate (2.5 g, 0.014 mmol) by heating under reflux for 30 min. The warm solution was acidified by HCl to pH 0 and the resulting precipitate collected after cooling. Recrystallization from DMF. Yield: 2.59 g (78%). M.p.  
30 330°C. UV (MeOH): 204 (4.54); [222 (4.37)]; 284 (4.17); 346 (4.25).

7-Hydroxy-3,6-dimethyl-1-phenyllumazine (37) [12]:

35 7-Hydroxy-6-phenyl-1,3-di-n-propyllumazine (38):

A suspension of 5,6-diamino-1,3-di-n-propyluracil (1.13 g, 0.005 mol) in H<sub>2</sub>O (30 ml), EtOH (5 ml) and AcOH (2 ml) was treated with ethyl phenylglyoxylate (1.25 g,

0.007 mmoles) and heated under reflux for 30 min forming a brownish oil. After cooling was acidified by HCl to pH 0 whereby the oil solidified. Filtration and recrystallization from EtOH/H<sub>2</sub>O gave yellowish needles.  
5 Yield: 1.28 g (75%). M.p. 245°C. UV (MeOH): 212 (4.30); [243 (4.01)]; 284 (4.02); 349 (4.19).

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## Materials and methods

Various models may be used for testing an immunosuppressive effect. In vivo, for example, different transplantation models are available. They are strongly influenced by different immunogenicities, depending on the donor and recipient species used and depending on the nature of the transplanted organ. The survival time of transplanted organs can thus be used to measure the suppression of the immune response. In vitro, there exist also various models. The most used are lymphocyte activation tests. Usually activation is measured via lymphocyte proliferation. Inhibition of proliferation thus always means immunosuppression under the experimental conditions applied. There exist different stimuli for lymphocyte activation:

- coculture of lymphocytes of different species (MLR = mixed lymphocyte reaction): lymphocytes expressing different minor and major antigens of the HLA-DR type (= alloantigens) activate each other non-specifically.

- CD3 assay: here there is an activation of the T-lymphocytes via an exogenously added antibody (OKT3). This antibody reacts against the CD3 molecule located on the lymphocyte membrane. This molecule has a costimulatory function. The interaction anti-CD3 (= OKT3)-CD3 results in T-cell activation which proceeds via the  $\text{Ca}^{2+}$ /calmodulin/calcineurin system and can be inhibited by CyA.

- CD28 assay: here specific activation of the T-lymphocyte goes also via an exogenously added antibody against the CD28 molecule. This molecule is also located on the lymphocyte membrane, and delivers strong costimulatory signals. This activation is  $\text{Ca}^{2+}$ -independent and thus cannot be inhibited by CyA.

## 35 Reagents

All derivatives were dissolved in 0.5 ml DMSO and further diluted in culture medium before use in in vitro

experiments. The culture medium consisted of RPMI-1640 + 10% FCS.

#### Mixed Lymphocyte Reaction

5 Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (Nycomed, Maorstua, Norway). Allogeneic PBMC or EBV-transformed human B cells [RPMI1788 (ATCC name CCL156)] which  
10 strongly express B7-1 and B7-2 were used as stimulator cells after irradiation with 30 Gy. MLR was performed in triplicate wells. After 5 days incubation at 37°C, 1 µCi [<sup>3</sup>H]-thymidine was added to each cup. After a further 16 hours incubation, cells were harvested and counted in a  
15 β-counter.

The percent suppression of proliferation by drugs was counted using the formula:

$$\text{Percent inhibition} = \frac{(\text{cpm+drugs}) - \text{cpm Cult. Med}}{(\text{cpm-drugs}) - \text{cpm Cult. Med}} \times 100$$

20

#### T cell purification

T cells were purified by removing non-T cells. Briefly, monocytes were removed by cold agglutination. The resulting lymphoid cells were further purified by a cell enrichment immunocolumn [Collect Human T (Biotex, Edmonton, Alberta, Canada)] by a process of negative selection. More than 95% of the B cells were removed with  
30 this procedure. After depletion, the resulting T cell preparation was highly purified explaining these cells could not be activated by PHA or rIL-2 alone at concentrations capable of stimulating RBMC prior to deletion.

35

Measurements of T cell proliferations induced by anti-CD3 mAb + PMA or anti-CD28 mAb + PMA

Highly purified T cells ( $10^6/\text{ml}$ ) were stimulated by immobilized anti-CD3 or anti-CD28 mAb in the presence of PMA. Anti-CD3 mAb (CLB-CD3; CLB, Amsterdam, The Netherlands) were fixed on the 96-microwell plates by incubating the wells with 50  $\mu\text{l}$  of mAb solution (1/800 dilution in culture medium). Anti-CD28 mAb (CLB-CD28; CLB, Amsterdam, The Netherlands) 50  $\mu\text{l}$  (1/650 dilution in culture medium) was added directly to the wells. Further, 20  $\mu\text{l}$  PMA (Sigma, St. Louis, MO, USA) solution (final concentration: 0.5 ng/ml) was added. Subsequently, 20  $\mu\text{l}$  of immunosuppressants were added by serial dilution in triplicate wells. Finally 100  $\mu\text{l}$  of the T cell suspension ( $10^6/\text{ml}$ ) was added. After 48-hour incubation at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$ , 20  $\mu\text{l}$  BrdU (100  $\mu\text{M}$  solution) (Cell Proliferation Elisa, Boehringer-Mannheim Belgium) was added to each well. After a further overnight incubation the T cell proliferation was measured using a colorimetric immunoassay for qualification of cell proliferation based on measurements of the incorporation of BrdU during DNA synthesis. The optical density (OD) was measured by a Behring EL311 plate reader at 450 nm (reference wavelength: 690 nm). The percent suppression of proliferation by drugs was counted using the formula:

$$\text{Percent inhibition} = \frac{(\text{OD+drugs}) - (\text{OD Cult. Med.})}{(\text{OD-drugs}) - (\text{OD Cult. Med.})} \times 100$$

In vitro immunosuppressive effect of Pteridine Derivatives as measured with the MLR and with tests involving polyclonal T cell proliferation induced by anti-CD3 mAb + PMA or anti-CD28 mAb + PMA (table II)

- In the table II column II shows the  $\text{IC}_{50}$  values of the various substances in the MLR. The  $\text{IC}_{50}$  value represents the lowest concentration of the substances that resulted in a 50% suppression of the MLR.

- Column III shows the  $\text{IC}_{50}$  value of the various substances for the anti-CD3 mAb + PMA pathway and row IV the  $\text{IC}_{50}$  values of the various substances for the

anti-CD28 mAb + PMA pathway.

- As a comparison the values of other immunosuppressants: CsA, FK506, Rapamycin, Leflunomide and Mycophenolic acid methatroxate (MTX) and 5-Fluoro-uracil (5-FU) in table III are given as well.

#### Whole Blood Assay (WBA)

WBA is a lymphoproliferation assay performed in vitro but using lymphocytes present in whole blood, taken from animals that were previously given test substances in vivo. Hence it reflects the in vivo effect of substances as assessed with an in vitro read-out assay.

Rats: inbred, male 6- to 8-weeks old R/A rats weighing  $\pm$  200 g were used as recipients.

Drug administration: Pteridine derivatives were dissolved in DMSO and further diluted with PBS. Products were given orally in different concentrations 2 times a day for 2 days. To perform the experiments, 6-8 hours after the last administration 1 ml of blood is taken by heart puncture after ether anesthesia and anticoagulated with 100 U/ml of preservative free heparine.

Whole Blood Assay: This assay was performed as we described previously [Use of the Methylxanthine Derivatives A802715 in Transplantation Immunology. II In vitro Experiments. (Yuan Lin, et al., Transplantation 1997, 63, No. 12, 1734-1738)].

Heparinized whole blood was diluted (1:25) with complete RPMI medium and stimulated with 15  $\mu$ g/ml of concanavalin A (Con A) in triplicate wells in 96-well microtiter plates at 37 °C and 5% CO<sub>2</sub>. After 96-h culture, proliferation was determined by measuring the incorporation (cpm) of [<sup>3</sup>H]-thymidine.

The Con A induced proliferation of lymphocytes taken from rats receiving the test substances (exp) was compared with that from rats receiving only the solvent (con). The percent suppression was calculated as follows:

## 5 Results

No.	%suppression	Administration of drugs	Blood taken after:
28	57%	80 mg/kg/d	8 h
34	63%	2x/d 2d 40 mg/kg/d 2x/d 2d	8 h

10 First, most of the pteridine classes (I) according to the  
invention contain compounds with a clear suppressive  
effect in the MLR (mixed lymphocyte reaction). The MLR is  
considered as an in vitro analogue of the transplant  
rejection as it is based on the recognition of allogeneic  
15 MHC (major histocompatibility antigens) on the stimulator  
leucocytes, by responding lymphocytes. Various established  
immunosuppressive drugs are known to suppress the MLR,  
and were also shown in this description.

From these data it can be deduced that the  
20 pteridine derivatives are effective in clinical  
situations where other immunosuppressants are active as  
well.

These include the prevention and/or treatment of  
organ transplant rejection, the prevention and/or  
25 treatment of both rejection and the occurrence of graft-  
versus-host-disease after BM transplantation; the  
prevention and/or treatment of autoimmune diseases  
including diabetes mellitus, multiple sclerosis,  
glomerulonephritis, rheumatoid arthritis, psoriasis  
30 systemic diseases such as vasculitis; scleroderma,  
polymyositis, autoimmune endocrine disorders  
(thyroiditis), ocular diseases (uveitis), inflammatory  
bowel diseases (Crohn's disease, colitis ulcerosa),  
autoimmune liver diseases (autoimmune hepatitis, primary

biliary cirrhosis) autoimmune pneumonitis and auto-immune carditis.

Whereas cyclosporine A and FK506 are only active in the anti-CD3 + PMA test, the pteridine derivatives according to the invention were active, not only in the anti-CD3 + PMA but also in the anti-CD28 + PMA test. It has been shown that the latter is Ca-calmodulin resistant, and resistant to CsA and FK506. The anti-CD28 + PMA pathway has also been called the cosignal pathway and is important to induce energy and even tolerance in T cells. Moreover, representative compounds have been found to be active in an whole blood assay.

Under the term "organ" in the description is understood all organs or parts of organs (even several) in mammals, in particular humans, for example kidney, heart, skin, liver, muscle, cornea, bone, bone marrow, lung, pancreas, intestine or stomach.

After organ transplantation, rejection of the transplanted organ by the recipient occurs (host-versus-graft reaction). After bone marrow transplantation, also rejection of the host by the grafted cell may occur (graft-versus-host reaction). Rejection reactions mean all reactions of the recipient body or of the transplanted organ which in the end lead to cell or tissue death in the transplanted organ or adversely affect the functional ability and viability of the transplanted organ or adversely affect the functional ability and viability of the transplanted organ or the recipient. In particular, this means acute and chronic rejection reactions.

Auto-immune disorders include, inter alia, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, pemphigus, atopic dermatitis, myositis, multiple sclerosis, nephrotic syndrome (in particular glomerulonephritis), ulcerative colitis or juvenile diabetes.

An additive or synergetic effect of pteridine derivatives and other immunosuppressants may be

anticipated. This may be especially, although not exclusively the case for combinations with CyA or FK 506 as the latter are not suppressive in the aCD28 pathway of T cell activation (table III) whereas most pteridine  
5 derivatives are.

The invention further relates to the use of cyclosporin A or FK506 or Rapamycine and at least one pteridine derivative according to the invention for the production of a pharmaceutical composition for inhibiting  
10 the replication of viruses such as picorna-, toga-, bunya-, orthomyxo-, paramyxo-, rhabdo-, retro-, arena-, hepatitis B-, hepatitis C-, hepatitis D-, adeno-, vaccinia-, papilloma-, herpes-, varicella-zoster-virus or human immunodeficiency virus (HIV); or for treating of  
15 cancer such as lung cancers, leukaemia, ovarian cancers, sarcoma, Kaposi's sarcoma, meningioma, colon cancers, lymph node tumors, glioblastoma multiforme, prostate cancers or skin carcinoses.

The invention further relates to the use of cyclosporin A or FK506 or rapamycin and at least one pteridine derivative of the general formula (I) for the production of a pharmaceutical composition for the treatment of human after organ transplantation or of  
20 (auto)immune disorders.

Hence, the advantage to associate pteridine with other immunosuppressants may be that, first, the therapeutic spectrum of action of the individual components is quantitatively and qualitatively broadened. Secondly that it allows, by means of a dose reduction  
25 without reduced efficacy but with increased safety, that the treatment of immune disorders which were hitherto no indication for immunosuppressive therapy as a result of side effects may be considered. At the same time, the therapy costs can be decreased to an appreciable extent.  
30

As a comparison, known pteridine derivatives are submitted to the same test conditions as the pteridine derivatives of the invention. These compounds and the results thereof are given in table IV and show no  
35

particular immunosuppressive activity.

In all tables

- 0: concentration > 151  $\mu\text{M}$
- 5   +: concentration range 16-150  $\mu\text{M}$
- ++: concentration range 1-15  $\mu\text{M}$
- +++: concentration range < 1  $\mu\text{M}$

The skilled person will appreciate that the preparation according to the invention may contain the pteridine compounds over a broad content range depending on the contemplated use of the preparation. Generally, the content of the preparation is within the range of 0.01-50 wt.%, preferably within the range of 0.01-10 wt.%, more preferably within the range of 0.1-10 wt.%, and most preferably within the range of 0.1-5 wt.%. Accordingly, the preparation may be used in a dosing regime which is suitable for most contemplated pharmaceutical utilities.

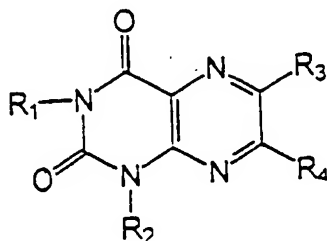
The preparation according to the invention may be used as such or in combination with any acceptable carrier material, excipient or diluent.

The preparation according to the invention may be administered orally or in any other suitable fashion. Oral administration is preferred and the preparation may have the form of a tablet, aqueous dispersion, dispersible powder or granule, emulsion, hard or soft capsule, syrup, elixir or gel. The dosing forms may be prepared using any method known in the art for manufacturing these pharmaceutical compositions and may comprise as additives sweeteners, flavoring agents, coloring agents, preservatives and the like. Carrier materials and excipients may include calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, binding agents and the like. The preparation may be included in a gelatin capsule mixed with any inert solid diluent or carrier material, or has the form of a soft gelatin capsule, in which the ingredient is mixed with a water or

oil medium. Aqueous dispersions may comprise the preparation in combination with a suspending agent, dispersing agent or wetting agent. Oil dispersions may comprise suspending agents such as a vegetable oil.

CLAIMS

1. Pharmaceutical composition for the treatment of autoimmuno disorders and/or for the treatment or prevention of transplant-rejections comprising a pteridine derivative of general formula:



wherein:

R<sub>1</sub> and R<sub>2</sub> are independently hydrogen; aliphatic saturated or unsaturated; straight or branched carbon chain with 1 to 7 carbon atoms; substituted or unsubstituted aryl or alkylaryl substituents, whereby the carbon atoms may be oxidized represented by alcohol or carbonyl function or carboxylic acids and their esters;

20

R<sub>3</sub> and R<sub>4</sub> are independently hydrogen, hydroxyl, halogen, alkyl, haloalkyl, alkoxy, wherein alkyl and the alkyl group may be branched or straight and contains one or four carbon atoms, formyl and derivatives such as hydroxylamino conjugates and acetals, cyano, carboxylic acids and carboxyl acid derivatives such as esters and amides, sulfhydryl, amino, alkylamino, cycloalkylamino, alkenylamino, alkynylamino, benzylamino, hydroxylalkylamino, morfolinoalkylamino, fenylhydrazino, morfoline, piperidine, mercaptobenzyl, mercaptoalkyl, cysteinyl ester, styryl, substituted or unsubstituted aromatic ring; aromatic or heterocyclic substituent substituted with an aliphatic spacer between the

30

pteridine ring and the aromatic substituent of 1 to 4 carbon atoms, whereby said spacer may contain an alcohol function, carbonyl function, halogen, ether, and may be saturated or unsaturated; branched or straight, saturated  
5 or unsaturated aliphatic chain of 1 to 7 carbon atoms which may contain one or more functions chosen from the group comprising carbonyl, alcohol, ether, carboxyester, nitro, thioalkyl, halogen or a pharmaceutically acceptable salt thereof; and

10

X and Y are independently oxygen or sulfur or a pharmaceutical salt thereof, and a pharmaceutically acceptable carrier.

15

2. Pharmaceutical composition according to claim 1, wherein:

$R_1$  and  $R_2$  are independently hydrogen, methyl, ethyl, propyl, isopropyl, cyclopropyl allyl and other  
20 alifatic and alicyclic saturated and unsaturated substituents with 1 to 5 carbon atoms;

3. Pharmaceutical composition according to claim 1-2,  
25 wherein:

$R_1$  and  $R_2$  are independently hydrogen; hydroxyl; halogen; alkoxy whereby the alkyl group being one to four carbon atoms; cyano; carboxyl acids and carboxyl acid  
30 derivatives such as esters and amides; sulfhydryl; substituted or unsubstituted aromatic or heterocyclic ring having as substituents one or more of following functions chosen from the group comprising: halogen, alkoxy, hydroxy, amino, alcohol, ether, nitro, alkyl,  
35 alkenyl, haloalkyl and haloalkenyl, cyano, carboxyl acids and derivatives; aromatic or heterocyclic substituent with an aliphatic spacer between the pteridine ring and the aromatic substituent of 1 to 4 carbon atoms, this

spacer may contain an alcohol function, carbonyl function, carboxyl, nitro, halogen, ether and may be saturated or unsaturated, branched or unbranched; branched or straight, saturated or unsaturated aliphatic chain of 1 to 7 carbon atoms which may contain one or more of following functions chosen from the group comprising: carbonyl, alcohol, ether, acetal, amino, imino, amino acid, carboxylester, cyano, nitro, thioalkyl, halogen; or a pharmaceutically acceptable salt thereof.

4. Pharmaceutical composition according to claim 1-3,

wherein:

15  $R_1$  and  $R_2$  are methyl.

5. Pharmaceutical composition according to claim 4, wherein  $R_4$  is hydrogen.

6. Pharmaceutical composition according to claim 5, wherein  $R_3$  is haloethyl or haloethylene substituted by phenyl or carboxylic acid ester; or is ethyl or ethylene substituted by nitro.

7. Pharmaceutical composition according to claim 4, wherein  $R_3$  is hydrogen.

8. Pharmaceutical composition according to claim 7, wherein  $R_4$  is haloethyl or haloethylene substituted by phenyl or carboxylic acid ester; or is chloro.

9. Pharmaceutical composition according to claim 4, wherein  $R_3$  is selected from cyano, carboxylic acid ester or dihalo methyl; and  $R_4$  is selected from chloro, amino  $C_4$ -alkyl, aminophenyl, thiomethylphenyl, thiomethyl-carboxylic acid ester.

10. Pharmaceutical composition according to any of the previous claims 1-9, wherein the pteridine derivative is a compound selected from the group comprising:

- 35 - 1,3-Dimethyl-6-(E)-styryllumazine (1)  
- 1,3-Dimethyl-6-[(E)-2-(pyrid-3-yl)vinyl]lumazine  
(2)

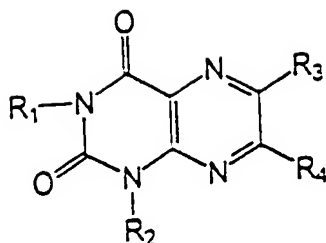
- 1,3-Dimethyl-6-[(E)-2-(pyrid-4-yl)vinyl]lumazine  
(3)
- 6-(1,2-Dibromo-2-phenylethyl)-1,3-dimethylumazine  
(4)
- 5 - 1,3-Dimethyl-6-[(E)-4-(phenyl)butadienyl]lumazine  
(5)
- 6-(1,2-Dibromo-2-(methoxycarbonyl)ethyl)-1,3-  
dimethylumazine (6)
- 6-(2-Bromo-2-methoxycarbonyl-ethenyl)-1,3-  
10 dimethylumazine (7)
- 6-[(2-Acetyl-2-ethoxycarbonyl)acetyl]-1,3-  
dimethylumazine (8)
- 6-[2,2-(Diethoxycarbonyl)acetyl]-1,3-  
dimethylumazine (9)
- 15 - 6-(1-Methoxy-2-methoxycarbonyl)ethenyl)-1,3-  
dimethylumazine (10)
- 1,3-Dimethyl-6-[(2-nitro)ethenyl]lumazine (11)
- 6-[(1-Hydroxy-2-nitro)ethyl]-1,3-dimethylumazine  
(12)
- 20 - 6-[(1-Ethylthio-2-nitro)ethyl]-1,3-  
dimethylumazine (13)
- 6-Hydroxymethyl-1,3-dimethylumazine (14)
- 1,3-Dimethyl-7-[(E)-2-(pyrid-2-yl)vinyl]lumazine  
(15)
- 25 - 1,3-Dimethyl-7-[(E)-2-(pyrid-3-yl)vinyl]lumazine  
(16)
- 1,3-Dimethyl-7-[(E)-2-(pyrid-4-yl)vinyl]lumazine  
(17)
- 1,3-Dimethyl-7-[(E)-4-(phenyl)butadienyl]lumazine  
30 (18)
- 7-[1,2-Dibromo-2-(methoxycarbonyl)ethyl)-1,3-  
dimethylumazine (19)
- 7-[(E)-2-methoxycarbonylethenyl]lumazine (20)
- 7-(1,2-Dibromo-2-phenylethyl)-1,3-dimethylumazine  
35 (21)
- 7-(1-Bromo-2-phenyl)ethenyl-1,3-dimethylumazine  
(22)
- 1,3-Dimethyl-6-(E)-styryllumazine (23)

- 7-Benzoyl-1,3-dimethylumazine (24)
- 7-Chloro-1,3-dimethylumazine (25)
- 1,3-Dimethyl-7-mercaptolumazine (26)
- 1,3-Dimethyl-6,7-diphenylumazine (27)
- 5 - 1,3-Dimethyl-6-phenyl-7-mercaptolumazine (28)
- 7-Methoxy-1,3-dimethyl-6-phenylumazine (29)
- 7-Chloro-1,3-dimethyl-6-phenylumazine (30)
- 6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-(4-methoxyphenyl)umazine (31a)
- 10 - 6-Benzoyl-1,3-dimethyl-7-(4-methoxyphenyl)umazine (31)
- 6-Benzoyl-7,8-dihydro-1,3-dimethyl-7-phenylumazine (32a)
- 6-Benzoyl-1,3-dimethyl-7-phenylumazine (32)
- 15 - 7-Methoxy-1,3-dimethyl-6-styryllumazine (33)
- 1-Methyl-6,7-diphenylumazine (34)
- 7-Hydroxy-3-methyl-6-phenylumazine (35)
- 7-Hydroxy-1,6-diphenylumazine (36)
- 7-Hydroxy-3,6-dimethyl-1-phenylumazine (37)
- 20 - 7-Hydroxy-6-phenyl-1,3-di-n-propylumazine (38).

11. Pharmaceutical composition containing a pteridine derivative according to claim 1-10 and a compound selected from the group comprising cyclosporine A, substituted xanthines, tacrolimus (FK506), Rapamycin (RPM), Leflunomide, Mofetil, adrenocortical steroids, cytotoxic drugs and antibody compositions as a combined preparation for simultaneous, separate or sequential use in the treatment of auto-immune disorders or of the prevention and/or treatment of transplant-rejection.

30 12. Method for treating auto-immune disorders or transplant-rejections in a subject by administering an effective amount of a pharmaceutical composition of claims 1-11.

13. Compound having the formula:



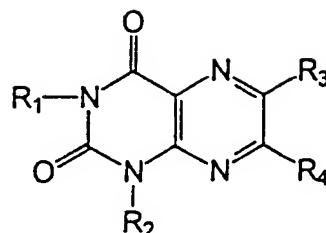
wherein:

R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are defined in claims 1-10.

10        14. Use of a compound as defined in claim 10 for  
the treatment of autoimmune disorders and/or the  
treatment and/or prevention of transplant rejections.

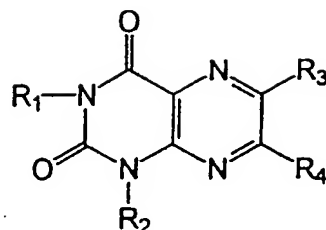
15        15. Method for selecting potent immunosuppressive  
agents based on the determination of the three parameters  
MLC, ACD<sub>1</sub> and ACD<sub>28</sub>.

Table I



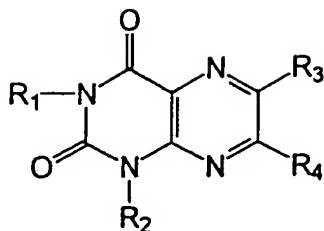
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1	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—CH=CH—}$	H
2	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—CH=CH—}$	H
3	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—CH=CH—}$	H
4	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—CHBr—CHBr—}$	H
5	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—(CH=CH)}_2\text{—}$	H
6	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—CHBr—CHBr—COOCH}_3$	H
7	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—CH=CH—COOCH}_3$	H
8	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—CO—CH—COOEt}$   COCH <sub>3</sub>	H
9	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—CO—CH—COOEt}$   COOEt	H
10	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—C=CH—COOCH}_3$   OCH <sub>3</sub>	H
11	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—CH=CH—NO}_2$	H
12	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—CHOH—CHNO}_2$	H
13	CH <sub>3</sub>	CH <sub>3</sub>	$\text{—CH—CH}_2\text{NO}_2$   SC <sub>2</sub> H <sub>5</sub>	H
14	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>2</sub> OH	H

Table I

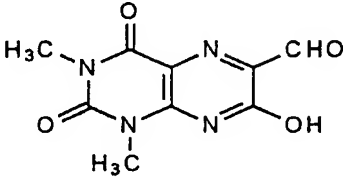
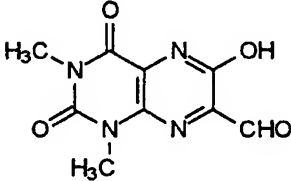
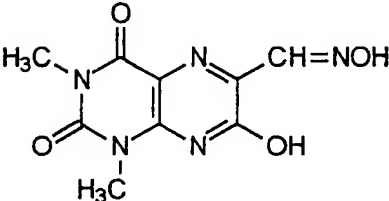
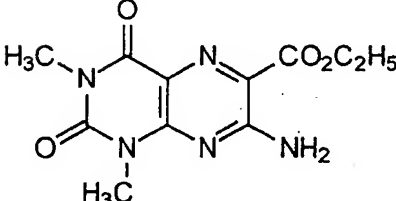
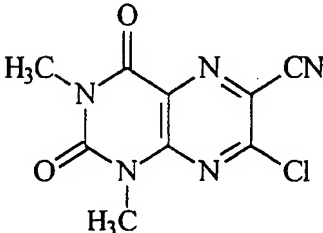


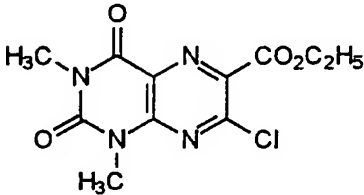
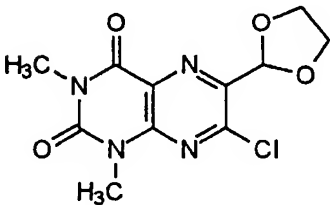
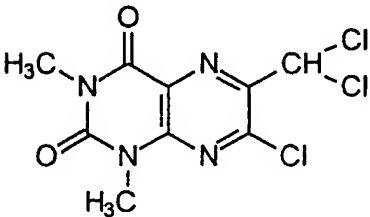
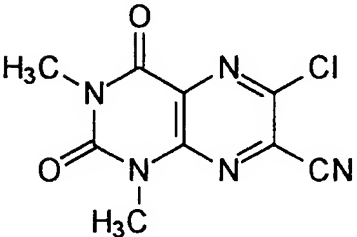
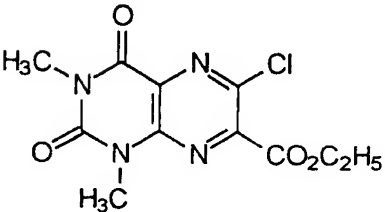
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15	CH <sub>3</sub>	CH <sub>3</sub>	H	
16	CH <sub>3</sub>	CH <sub>3</sub>	H	
17	CH <sub>3</sub>	CH <sub>3</sub>	H	
18	CH <sub>3</sub>	CH <sub>3</sub>	H	
19	CH <sub>3</sub>	CH <sub>3</sub>	H	
20	CH <sub>3</sub>	CH <sub>3</sub>	H	
21	CH <sub>3</sub>	CH <sub>3</sub>	H	
22	CH <sub>3</sub>	CH <sub>3</sub>	H	
23	CH <sub>3</sub>	CH <sub>3</sub>	H	
24	CH <sub>3</sub>	CH <sub>3</sub>	H	
25	CH <sub>3</sub>	CH <sub>3</sub>	H	Cl
26	CH <sub>3</sub>	CH <sub>3</sub>	H	SH

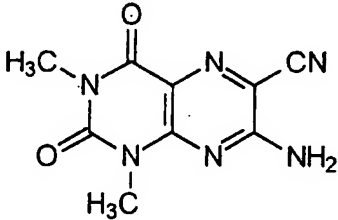
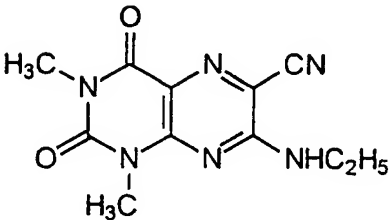
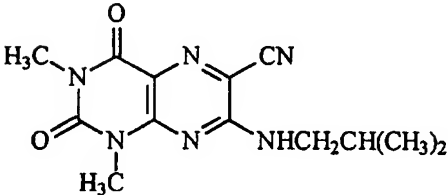
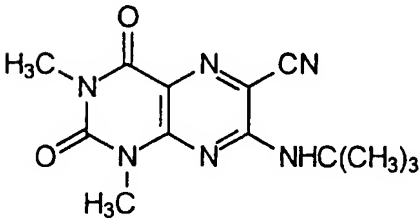
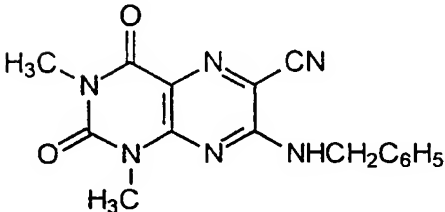
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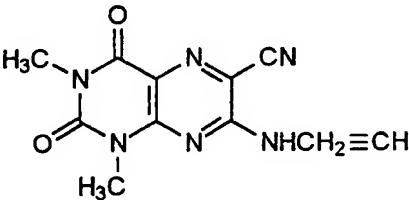
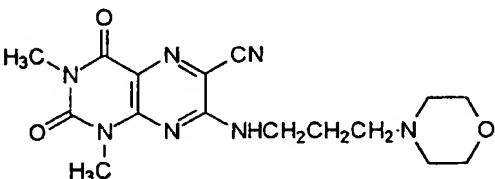
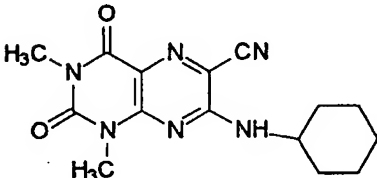
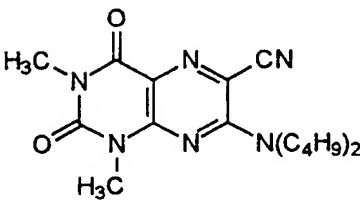
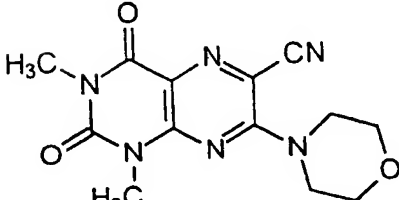


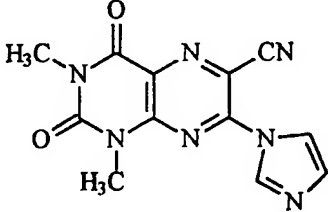
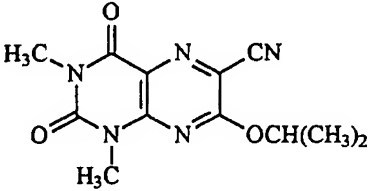
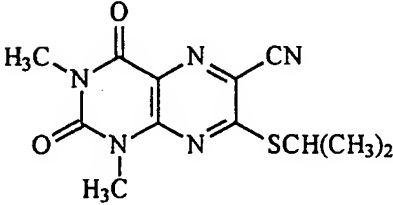
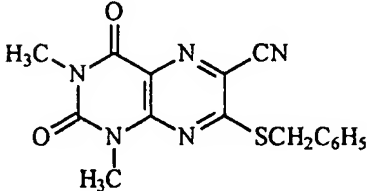
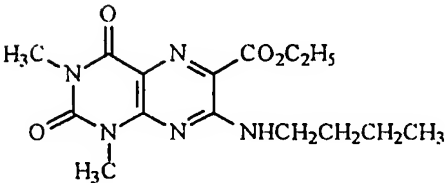
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28	CH <sub>3</sub>	CH <sub>3</sub>		SH
29	CH <sub>3</sub>	CH <sub>3</sub>		OCH <sub>3</sub>
30	CH <sub>3</sub>	CH <sub>3</sub>		Cl
31	CH <sub>3</sub>	CH <sub>3</sub>		
32	CH <sub>3</sub>	CH <sub>3</sub>		
33	CH <sub>3</sub>	CH <sub>3</sub>		OCH <sub>3</sub>
34	H	CH <sub>3</sub>		
35	CH <sub>3</sub>	H		OH
36	H			OH
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38	Pr	Pr		OH

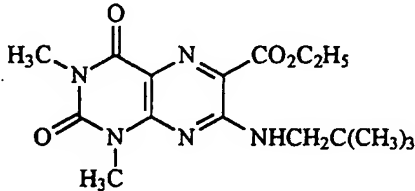
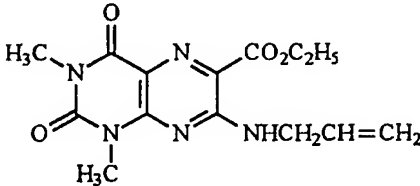
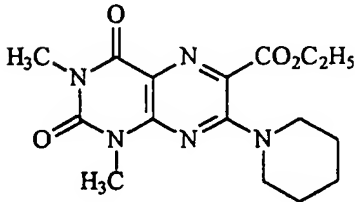
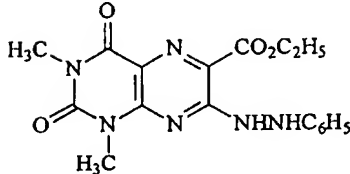
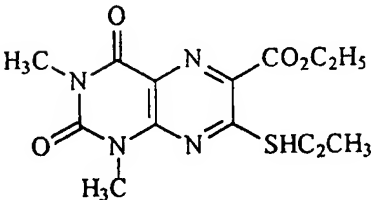
Compound n°	MLR	aCD3	aCD2 8
39			
	0	++	++
40			
	+	+	+
41			
	0	0	0
42			
	0	0	0
43			
	+	++	++

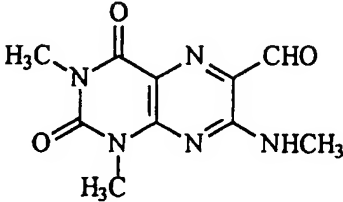
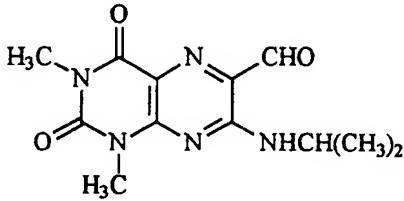
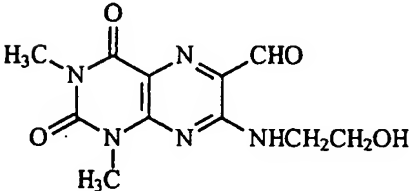
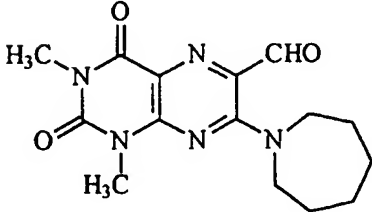
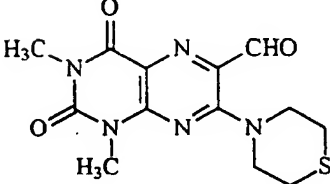
Compound n°	MLR	aCD3	aCD2 8
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45			
46			
47			
48			

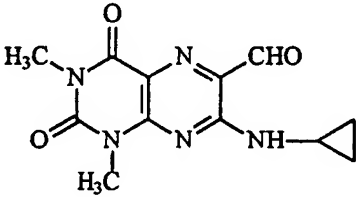
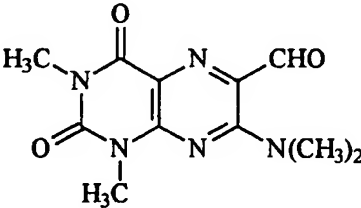
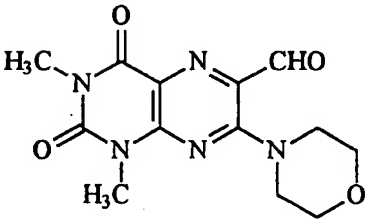
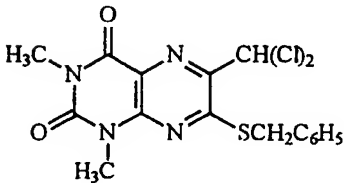
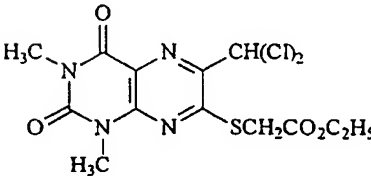
Compound n°	MLR	aCD3	aCD2 8
49			
	0	0	0
50			
	0	0	0
51			
	+	+	+
52			
	+	+	0
53			
	+	+	+

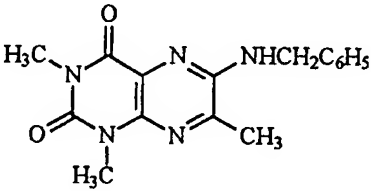
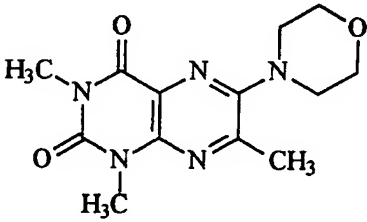
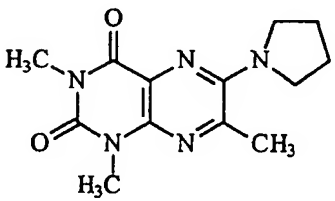
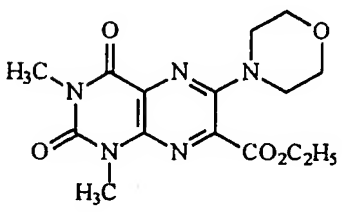
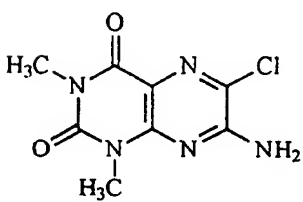
Compound n°	MLR	aCD3	aCD2 8
54			
	+	+	+
55			
	0	0	+
56			
	++	+	+
57			
	++	0	+
58			
	0	0	0

Compound n°	MLR	aCD3	aCD2 8
59			
	+	++	++
60			
	0	0	0
61			
	+	+	+
62			
	0	++	++
63			
	++	+	+

Compound n°	MLR	aCD3	aCD2 8
64			
	+	+	+
65			
	+	+	+
66			
	+	+	+
67			
	+	+	++
68			
	+	+	+

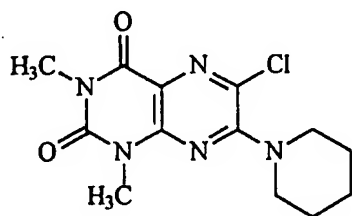
Compound n°	MLR	aCD3	aCD2 8
69			
	+	0	+
70			
	+	+	+
71			
	+	+	+
72			
	+	+	+
73			
	+	+	+

Compound n°	MLR	aCD3	aCD2 8
74			
	+	+	+
75			
	0	0	+
76			
	0	+	+
77			
	+	++	++
78			
	+	++	++

Compound n°	MLR	aCD3	aCD2 8
79			
	+	+	+
80			
	0	+	+
81			
	+	+	+
82			
	+	+	+
83			
	0	0	0

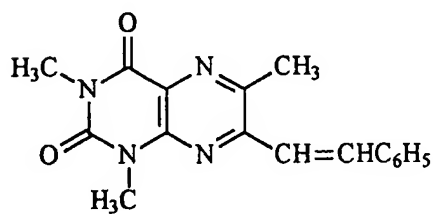
Compound n°	MLR	aCD3	aCD2 8
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84



0 + +

85



+ + +

Table II

Compound n°	MLR	aCD3	aCD28
1	75	> 200	> 200
2	30	110	110
3	20	150	150
4	15	3.4	1.55
5	75	140	140
6	12	0.55	0.08
7	4.2	0.58	0.08
8	> 200	80	20
9	> 200	125	90
10	70	80	70
11	12	0.8	0.4
12	90	25	15
13	4.5	0.35	0.09
14	> 200	> 200	25

Table II

IC<sub>50</sub> in  $\mu$ M of pteridine derivative

Compound n°	MLR	ACD3	aCD28
15	125	125	80
16	20	100	60
17	75	125	100
18	30	110	80
19	1.9	0.5	0.5
20	15	12	12
21	12	0.6	0.1
22	2.7	2.4	2.7
23	50	115	115
24	120	120	80
25	12	0.5	0.1
26	60	35	25

Table II

IC<sub>50</sub> in  $\mu$ M of pteridine derivative

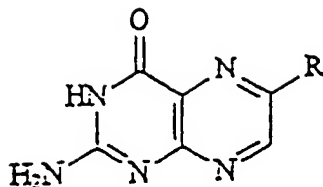
Compound n°	MLR	aCD3	aCD28
27	> 200	85	100
28	25	25	25
29	40	100	70
30	3.75	0.08	0.07
31	150	90	90
32	> 200	75	75
33	20	> 200	> 200
34	35	25	25
35	55	20	25
36	> 200	75	110
37	65	180	> 200
38	120	80	70

Table III

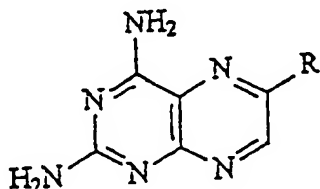
I.S.	IC50		
	Immunosuppressant		
	MLR	aCD3	aCD28
CyA	20 nM	50 nM	N.S.
FK506	1 nM	1 nM	N.S.
Rapamycin	1 nM	1 nM	1 nM
Leflunomide	25 $\mu$ M	15 $\mu$ M	20 $\mu$ M
Mofetil	<0.5 $\mu$ M	50 nM	50 nM
MTX	10 $\mu$ M	>200 $\mu$ M	> 200 $\mu$ M
5-FU		50 $\mu$ M	17 $\mu$ M

N.S. = not suppressive even not in the highest  
Concentration

Table IV



R	MLR	aCD3	aCD28
CH <sub>2</sub> OOCOCH <sub>3</sub>	>200	>200	>200
CH <sub>2</sub> OC <sub>6</sub> H <sub>5</sub>	>200	>200	>200
CH <sub>2</sub> NHCH <sub>3</sub>	>200	>200	>200
CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	>200	>200	>200
CH <sub>2</sub> S CH <sub>3</sub>	>200	>200	>200



R	MLR	aCD3	aCD28
CH <sub>2</sub> S CH <sub>3</sub>	>200	>200	>200
CH <sub>2</sub> O CH <sub>6</sub> (CH <sub>3</sub> ) <sub>2</sub>	>200	>200	>200
CH <sub>2</sub> O CH <sub>3</sub>	>200	>200	>200
CH <sub>2</sub> O CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	>200	>200	>200
CH <sub>2</sub> NH CO CH (CH <sub>3</sub> ) <sub>2</sub>	>200	>200	>200